Plant hormones: **Dissecting the gibberellin response pathway**

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The recent cloning of three *Arabidopsis* genes that regulate the response to gibberellin – one of the five 'classical' plant hormones – provides the first glimpse of possible molecular mechanisms operating in gibberellin signal transduction in plants.

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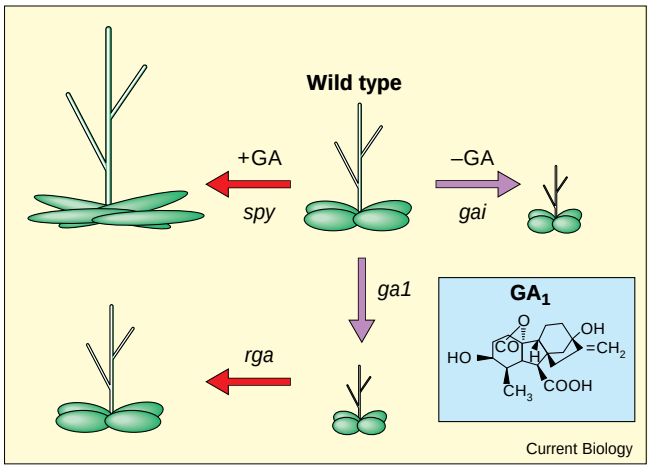
Plant development is amazingly plastic — for example, identical pine seeds can give rise to a majestic tree in a forest or a scraggly bush on a rocky cliff. How is plant growth and development regulated so as to be capable of achieving such dramatically different endpoints? One factor that has emerged as a key regulator of plant form is gibberellin (GA; see Figure 1 inset for the chemical structure). GA acts in both a continuous and a discrete manner during the life cycle of a plant. During the entire life cycle, GA promotes cell elongation; this role is particularly important given that plant form is entirely dictated by cell elongation and cell division in the absence of cell mobility. GA also acts as a regulator of key transition points in the plant life cycle by its ability to promote the germination of seeds and induction of flowering. The characterization of GA signaling pathways would greatly facilitate an understanding of how GA regulates these diverse processes.

Over the years, a substantial collection of mutants that appear to be defective in GA signaling has been isolated from a variety of species [1]. Most of these mutants fall into either of two classes: those that resemble GA-deficient plants, but do not respond to exogenous application of GA; and those that resemble plants in which a GA response pathway has been constitutively activated. Identification of such mutants has not been a solely academic enterprise. *Rht* mutant wheat plants fall into the first class; *Rht* mutations are the genetic basis of the high-yielding wheat varieties that contributed to the 'green revolution'.

The role of GA in *Arabidopsis* growth and development has been extensively characterized at the phenotypic level and through genetic analysis. As in other plants, GA promotes germination, shoot elongation and flowering in *Arabidopsis*. The GA biosynthetic pathway has been well characterized, and several GA biosynthetic genes have been cloned. *Arabidopsis* GA signaling mutants have been identified that fall into both of the two classes described above (Figure 1). Thus, the *gai* mutation leads to a GA-unresponsive dwarf plant, whereas the *spy* and *rga* mutations each lead to plants in which a GA response pathway appears constitutively activated. Recently, the wild-type genes corresponding to all three of these loci have been cloned [2–4]. These advances provide a significant starting point for understanding GA signal transduction.

SPINDLY (*SPY*) was the first of the three genes to be cloned. The recessive *spy* mutations were identified when mutagenized seeds were screened for the ability to germinate in the presence of paclobutrazol, a GA biosynthetic inhibitor [5]. The *spy* mutations can partially suppress all of the GA-deficient phenotypes associated with a mutation in *GA1*, which encodes an enzyme that catalyzes the first committed step of GA biosynthesis. Thus, the genetic evidence suggests that *SPY* encodes a negative regulator of GA signal transduction that acts early in the pathway. It is important to note that *spy* plants are still GA responsive: a *spy* plant treated with exogenous GA becomes taller, suggesting either that SPY may not be directly in the GA signaling pathway, or that there are multiple pathways for GA signal transduction.

Figure 1



The various types of *Arabidopsis* mutant with altered responses to GA that are discussed in the text. The inset shows the chemical structure of GA_1, one particular type of GA. In wild-type plants, GA promotes germination, shoot elongation and the initiation of flowering, among other roles. The *spy* mutation phenocopies the effect of spraying plants with GA. The *gai* mutation phenocopies the effect of treating plants with a GA biosynthetic inhibitor. The *ga1* mutation disrupts GA biosynthesis. The *rga* mutation suppresses the GA-deficient phenotypes associated with the *ga1* mutation. A null allele of *GAI*, and all known mutant *RGA* alleles, have only a slight phenotype in a wild-type background.

Jacobsen *et al.* [2] cloned *SPY* via the identification of a mutant allele tagged by insertion of T-DNA from the *Agrobacterium tumefaciens* Ti plasmid. When *SPY* was cloned, it was noted that the open-reading frame includes a sequence encoding several tandem repeats of the tetratricopeptide repeat (TPR) motif that in other contexts is known to mediate protein–protein interactions. Subsequently, a number of genes have been cloned that encode enzymes that catalyze the *O*-linked glycosylation of serine and threonine residues with *N*-acetylglucosamine, and thus are known as *O*-GlcNAc transferases [6,7]. The SPY protein sequence shows similarities to those of *O*-GlcNAc transferases, suggesting that SPY itself is an *O*-GlcNAc transferase.

Evidence has been accumulating that *O*-GlcNAc modification of proteins serves a regulatory function, either as a direct consequence of adding the *O*-linked GlcNAc group onto a target protein, or by competition for phosphorylation [6,7]. Modification of proteins by *O*-GlcNAc groups appears to be as frequent as phosphorylation, and the modification itself can be a dynamic event, suggesting a regulatory function. Identification of *spy* mutants may, thus, provide the first genetic evidence that an *O*-GlcNAc transferase really does act as a regulator in a signaling pathway. SPY is predicted to be an *O*-GlcNAc transferase whose ability to interact with its substrates is mediated by the TPR motif. Because loss of SPY function leads to an increase in GA responsiveness, it is hypothesized that *O*-GlcNAcylation of a GA signaling component by SPY acts to decrease GA signal transduction.

The *GIBBERELLIN INSENSITIVE* (*GAI*) gene was identified during characterization of mutant plants with a GA-deficient phenotype, one of which, *gai*, was found to be unresponsive to exogenous application of GA [8]. The *gai* mutation is semi-dominant — heterozygotes have a mutant phenotype, but not so severe as that of homozygotes. The *gai* mutant plants mimic all aspects of the GA-deficient phenotype, suggesting that GAI, like SPY, acts early in the GA signal transduction pathway. It is important to note that, although *gai* plants are GA-nonresponsive, they are still GA-dependent; for example, *gai* plants still require GA biosynthesis for germination.

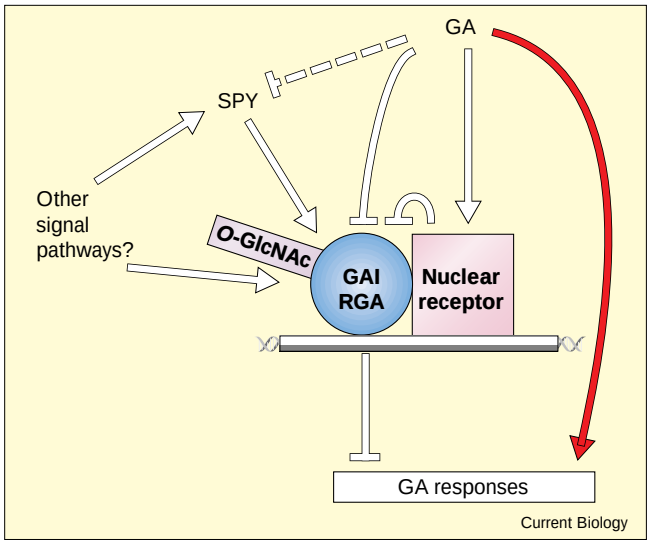
Peng *et al.* [3] cloned *GAI* by using a probe for the transposable element that inserted into the *gai* allele. A nearly identical gene, named *GRS* for '*GAI-related sequence*', was also cloned by using the *GAI* gene as a heterologous probe. The encoded protein sequences show that GAI and GRS are members of a family of probable transcription factors, the so-called VHIID family. GAI and GRS contain leucine heptad repeats, a putative nuclear localization signal, and an LXXLL motif that has recently been demonstrated to mediate interaction of transcriptional co-activators with nuclear receptors [9]. In the case of the semi-dominant *gai-1* allele, an in-frame deletion removes 17 amino acids from near the amino-terminus of the encoded protein. In addition, a putative *gai* null allele exhibits a weak *spy*-like phenotype, indicating that wild-type GAI functions as a repressor of GA signaling. Peng *et al.* [3] propose that GAI is directly inactivated by GA, and that the *gai-1* mutation results in a dominant gain-of-function mutant protein that is insensitive to GA and constitutively downregulates GA signal transduction.

The *REPRESSOR OF GA1-3* (*RGA*) gene was identified in a screen in which *ga1* mutant plants — which are defective in GA biosynthesis — were screened for second-site mutations that alleviate their GA-deficient phenotype (for example, that show reduced dwarfing) [10]. Both *spy* and *rga* mutants were identified in the screen; like *spy* mutations, the *rga* mutations are recessive. The *rga* mutations do not, however, suppress as many aspects of the GA-deficient phenotype as *spy* mutations; most notably, they do not suppress the defect in germination. Furthermore, a *spy rga* double mutant exhibits additive phenotypes, suggesting that SPY and RGA are involved in separate GA signal transduction pathways.

The cloning of *RGA* by Silverstone *et al.* [4] showed that it is identical to the *GRS* gene described above. *RGA* was cloned by genomic subtraction, looking for wild-type DNA sequences that do not hybridise to DNA from plants with *rga* deletion alleles. Consistent with the hypothesis that RGA and GAI are transcription factors, an RGA–GFP fusion protein has a nuclear localization in onion epithelial cells. Like *gai* null alleles, *rga* mutant alleles cause a very weak phenotype in a wild-type background. *RGA* and *GAI* are both ubiquitously expressed in all parts of the plant that have been examined. The implication is that GAI and RGA are partially redundant repressors of GA signaling that may act separately and/or jointly to downregulate GA responses. It will be interesting, as suggested by Silverstone *et al.* [4], to isolate plants defective in both *GAI* and *RGA* and determine how extensively GA signaling is upregulated in an otherwise wild-type background; if the double mutants do not have a severe phenotype, it would suggest the existence of other factors that convey the GA signal.

Several models can be constructed to fit the available data, one of which is illustrated in Figure 2. The GA signal could travel along one or more of three routes. The simplest scenario is that GA directly inactivates GAI and RGA, thus promoting GA responses. The LXXLL motifs in GAI and RGA, however, suggest that they may interact with a nuclear receptor complex. An alternative possibility, consequently, is that GA activates a transcription factor analogous to steroid receptors, which then directly inhibits GAI and RGA. Finally, it is quite possible that GA signal transduction is mediated by additional pathways that are independent of GAI and RGA. This possibility is

Figure 2



A model of how SPY, GAI and RGA may act in GA signal transduction, based on genetic data and protein sequence similarity. SPY is predicted to be an *O*-GlcNAc transferase, whereas GAI and RGA are predicted to be nearly identical transcriptional regulators. It is not known whether GAI and RGA are transcriptional activators or repressors. Arrows denote activation, bars denote repression. The square represents a hypothetical steroid-like nuclear receptor. The dashed bar from GA to SPY indicates that SPY activity may be directly repressed by the GA signal. SPY is likely to act on proteins in addition to GAI and RGA that regulate GA signal transduction. The red arrow indicates the possibility that some GA signal transduction pathways may be independent of GAI and RGA.

especially likely in light of the observation that *rga* mutations do not alleviate all of the phenotypes associated with a deficiency of GA. SPY may directly activate GAI and/or RGA through *O*-GlcNAcylation and may act on other GA signaling components as well. The observation that *spy* is epistatic to *gai* is consistent with SPY having a direct role in GAI action [2,3]. What is the role of SPY, GAI and RGA if they are not directly on the GA signal transduction pathway? GA responses are known to be modified by other environmental factors, most notably light, so these three proteins could be signaling integrators that modify GA responsiveness as a function of inputs from other signaling pathways.

All three of the genes involved in regulating the GA response that have been cloned so far seem to encode negative regulators of GA signaling. Where are the positive acting factors of the GA response pathway? One candidate positive regulator of GA signaling is the product of the *PICKLE* (*PKL*) gene [11]. Unlike *gai*, the *pkl* mutation is recessive; the phenotype of *pkl* mutants resembles that of GA-deficient plants and is partially corrected by exogenous application of GA. The *pkl gai* double mutant plants show a strong, synergistic GA-deficient phenotype, suggesting that PKL and GAI are involved in separate GA signaling pathways. Identification of the role of PKL, if any, in GA signaling awaits further phenotypic characterization of *pkl* plants and cloning of the *PKL* gene.

The cloning of *SPY*, *GAI* and *RGA* marks the first molecular characterization of components of the GA signaling pathway. Some of the more obvious experiments that are now possible include identification of substrates of SPY and genes that are targets of GAI and RGA. Identification of these and other components of GA signal transduction should lead to a greater understanding of the role of GA in plant development, and of how the remarkable developmental plasticity of plants is achieved. It is worth noting that cloning of *GAI* and *RGA* may lead to some immediate agricultural applications. The *gai* mutation is analogous in certain respects to the *Rht* mutation of high-yielding wheat mentioned at the start of this article. *GAI* homologs in crop species (a putative rice homolog has already been identified from the EST database) could be engineered to carry a *gai*-like mutation — perhaps by making transgenic plants carrying dominant gain-of-function mutant versions of *GAI* and *RGA* — in an attempt to alter the harvest index of the crop.

References

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